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Experimental Photoallergic Contact Dermatitis: A Mouse Model

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We have induced photoallergic contact dermatitis in mice to 3,3′,4′,5 tetrachlorosalicylanilide (TCSA), chlorpromazine and 6-methylcoumarin. These compounds are known to produce photoallergic contact dermatitis in humans. The photoallergic contact dermatitis reaction in the mouse is immunologically specific viz. mice photosensitized to TCSA react, by photochallenge, to that compound and not to chlorpromazine, and conversely. The reaction requires UVA at both sensitization and challenge. It appears to be T-cell mediated in that it can be passively transferred to syngeneic mice by lymph node cells from actively sensitized mice, the histology of the reactions resembles that of classic allergic contact dermatitis in mice, challenge reactions are seen at 24 but not at 4 hr, and photoallergic contact dermatitis can be induced in B-cell deficient mice. The availability of a mouse model for the study of photo-ACD will facilitate the identification of pertinent control mechanisms and may aid in the management of the disease. It is likely that a bioassay for photoallergens of humans can be based on this mouse model.

It is generally believed that photoallergic contact dermatitis (photo-ACD) is an immunologic response of delayed-type hypersensitivity [1–3]. This view has evolved primarily from clinical observations of sensitized patients and from limited experimental work involving the induction of photosensitivity in guinea pigs and humans. These studies have emphasized the clinical and histological similarities between photo-ACD and classical allergic contact dermatitis [2].

Objective experimental evidence for the role of immunological mechanisms in photo-ACD is limited. Indeed, the existing evidence has been criticized [4]. The mechanisms by which ultraviolet radiation (UVR) provokes the response is uncertain, although it has been postulated that photon absorption by the sensitizer and the ensuing photochemical reaction are somehow responsible for the formation of the haptene or immunogen [5]. Several other explanations for the role of UVR have been suggested [4].

In a limited study, Harber and Shalita reported the successful passive transfer of photocontact sensitivity to TCSA (3,3′,4′,5 tetrachlorosalicylanilide) with peritoneal exudate cells from actively sensitized Hartley guinea pigs to 3 of 5 naive Hartley guinea pigs [6]. Herman and Sams observed that peritoneal exudate cells derived from guinea pigs putatively photosensitized to TCSA failed to migrate in the presence of a TCSA-albumin conjugate prepared by exposing an aqueous solution, that contained both TCSA and albumin, to UVR [7]. Irradiated TCSA or irradiated albumin alone failed to give this effect. These findings suggested that the production of macrophage inhibitory factor (MIF) by sensitized cells was a specific response.

The aim of the present investigation was to develop a simple animal model suitable for studying the basic mechanisms governing photo-ACD. We shall describe the production of photo-ACD in the mouse and provide evidence that it is a reaction of delayed-type hypersensitivity (DTH). The mouse provides an advantageous experimental model since its immunological and genetic parameters are very well defined. In addition during the past several years, the regulatory mechanisms controlling the induction and expression of classical allergic contact dermatitis have been under intensive study in the mouse; thus, there is a large data base dealing with the regulation of murine ACD [8–11]. The results of studies of photo-ACD in a mouse model can be compared with these results.

MATERIALS AND METHODS

UVR Sources

The UVB source was a bank of four FS-20 sunlamp fluorescent tubes. These bulbs emit a continuous spectrum extending from about 280 nm to 380 nm, with a peak at around 313 nm. A bank of 4 fluorescent blacklight tubes (F-20 BL) served as the main source of UVA energy. The spectrum extends from 300 nm to about 400 nm, peaking at 360 nm. Irradiance was measured with calibrated cosine-corrected photodiode-type detectors using an International Light model IL700 research radiometer. For UVB, a model SEE-240 detector equipped with an interference filter with spectral response from 270 to 320 nm (peak response at 297 nm) was used. The UVA detector was a model SEE-010 photodiode with a spectral response from 300 to 400 nm (peak response at 365 nm). UVB irradiance at skin level was 0.36 mW/cm^2 and UVA irradiance 1.44 mW/cm^2.

Mice

CBA/J, Balb/c, C57Bl/6 and DBA/2 mice were purchased from the Jackson Laboratory (Bar Harbor, Maine). ICR outbred albino mice were purchased from the Institute for Cancer Research (Fox Chase, PA). C57Bl/6 × CBA/J F-1 mice were bred from stock secured from the Jackson Laboratory; breeding pairs were set up in individual cages in our animal room.

All mice were housed in plastic cages with wood shavings for bedding. In the case of the breeding pairs, cages were individually covered with glass wool bonnets; this practice was usually, but not always, followed with the other mice. The mice were fed fresh Purina Mouse Chow and had unlimited access to acidified water (pH about 3.0). Antibiotics were not used. The mouse colony was kept in a temperature-controlled, light-cycled room with care conforming to NIH guidelines.

Chemicals

TCSA (3,3′,4′,5 tetrachlorosalicylanilide) was secured from Hexcel Fine Organics (Lodi, NJ); Chlorpromazine was obtained from Smith Kline and French (Philadelphia); 6-methylcoumarin was a gift from Dr. D. L. Opdyke of the Research Institute of Fragrance Materials, and Sodium Omadine was obtained from Olin Mathieson Chemical Corporation (New York, NY). The vehicle for all of the photoallergens was a solution consisting of 4 parts acetone and 1 part corn oil. A preparation of heat-killed *C. parvum*, originating from cultures derived from strain #11827 of the American Type Culture Collection, was a gift from Dr. Irving Millman (Fox Chase Cancer Center, PA) [12]. Cyclophosphamide was purchased from Sigma Chemicals (St. Louis).

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Abbreviations:
- DTH: delayed type hypersensitivity
- MIF: macrophage inhibitory factor
- TCSA: 3,3′,4′,5 tetrachlorosalicylanilide

B-cell Deficient Mice

C57Bl/6 × CBA/J mice were rendered B-cell deficient by the chronic administration, beginning within 24 hr of birth, of a goat antisera directed against mouse IgM [13,14]. Mice so treated were deemed B-cell deficient by the following criteria: (1) as determined by precipitation in gel, their serum lacked mouse IgM and contained goat anti-mouse IgM, (2) by direct immunofluorescence, all of their spleen cells and their lymph node cells lacked surface immunoglobulin, in contrast to the spleen cells and lymph node cells of normal mice which had a substantial proportion of their nucleated cells with surface immunoglobulin, (3) their spleen cells failed to respond in culture to the B-cell mitogen LPS but did respond normally to the T-cell mitogen Con-A, and (4) in contrast to normal mice, who developed high titers of specific antibody, they failed to make any detectable antibody response to sheep red blood cells (hemagglutination, hemolysis in gel) or to DNP-Keyhold Limpet Hemocyanin in Freund's complete adjuvant (radioimmune assay using Protein A and, as antigen, DNP-human serum albumin). These findings relative to the B-cell deficient mouse model accord with the reports from other laboratories that use mice made B-cell deficient in the same way [15,16].

Photosensitization

The mice were anesthetized with pentobarbital and fixed in place by taping their feet to a wooden board. The photoallergen was pipetted onto a clipped site on the flank and the site irradiated 30 minutes later with UVB (0.1 joules/per cm^2) followed by UVA (5 joules/per cm^2). During the UVR (UVB, UVA) exposure, the ears of the mice were protected with several layers of paper toweling.

Challenge was done in the following way. The mice were anesthetized with pentobarbital and a baseline reading taken of the thickness of their test ear using an engineer's micrometer bearing a rachet. Then, 0.1 ml of test chemical was applied and the ears exposed to UVA (5 joules/cm^2). Readings were made at 24 and 48 hr of the thickness of the test ears from the experimental mice, and from control mice tested in parallel. (In a few cases, 4-hr readings were taken; however, this was not done routinely since the procedure of measurement of ear thickness the same day as challenge was too stressful and resulted in considerable morbidity and some mortality of the test mice.) The methodology is essentially that used by many investigators for the study of classical ACD in mice, the variations introduced being those required for the activation of the process by ultraviolet light.

Passive Transfer of Photo-ACD

Donor mice were sensitized by a single application of 0.01 ml of 1% TCSA in a 4:1 solution of acetone:corn oil to each of their 4 clipped dorsal quadrants followed, 30 min later, by irradiation of the sensitization site with UVB (0.1 joules/cm^2) and UVA (5 joules/cm^2). This was repeated Day 1, and, additionally *C. parvum* (30 μg) was injected intradermally into a rear quadrant. The *C. parvum* immunoadjuvant was utilized so as to have more strongly sensitized donors. Four days later the mice were sacrificed and the lymph nodes draining the four sensitization sites were removed and a single cell suspension of lymph node cells made in RPMI media containing 10% fetal calf sera. Red blood cells were lysed with ammonium chloride and the cell suspension washed 3 times. A cell count was made, utilizing trypan blue uptake to identify nonviable cells. Recipient mice were injected via tail vein with the cells and ear tested for photo-ACD 1 hr later.

Histology

Ear specimens from experimental and control mice, secured at the termination of particular experiments were preserved in 10% buffered formalin and processed to hemotoxylin-eosin slides by routine procedures.

Statistics

The significance of differences between study groups in particular experiments were analyzed by a one-tailed Mann Whitney U-test, $p \leq 0.02$ being considered significant [17].

RESULTS

Photosensitization to TCSA

In a typical experiment, 2 groups (I and II) of 6 ICR mice were injected intraperitoneally with cyclophosphamide (150 mg/k) on Day −3. On Day 0 and Day 1 these animals were photosensitized to TCSA and, along with 2 groups (III and IV) of control animals, were ear-challenged on Day 7. Readings of the challenge sites were made at 24 and 48 hr. The protocol and results are shown in Table I. Specimens from challenge reactions of representative mice were taken for histopathological study after the 48-hr measurements (Figure). A comparison of Groups I and II demonstrate that UVA at Day 7 challenge is necessary to elicit the photosensitivity; 1 of the 6 mice in Group II had increased ear thickening with TCSA challenge alone (11 units at 24 hr, 9 units at 48 hr) suggesting classic sensitivity to TCSA in that animal. In Table I, the slightly

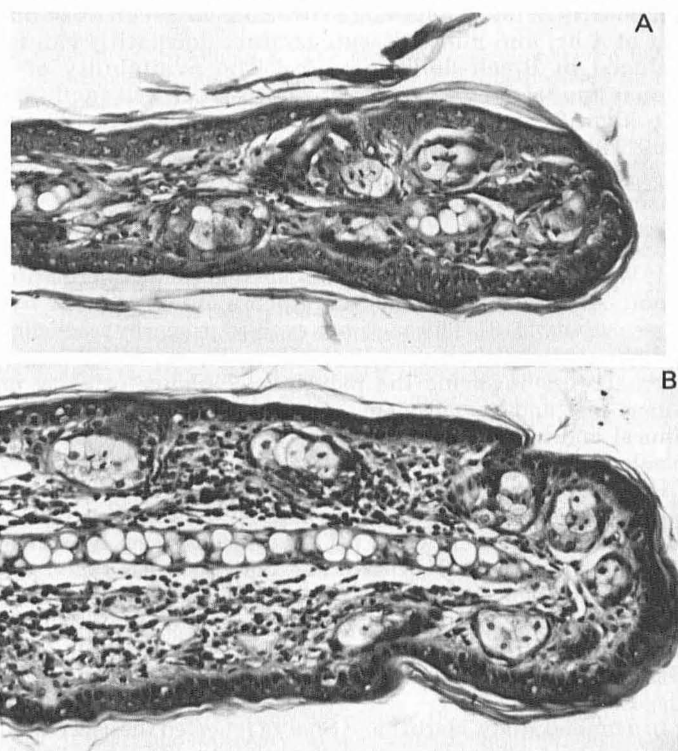

FIG 1. Photo-ACD was induced in ICR albino mice as outlined in Table I. The histology of a typical 48-hr reaction from a Group III control mouse (Fig 1*A*) and from a Group I experimental mouse (Fig 1*B*) are shown. The TCSA photo-ACD reaction at 48 hr shows edema and a dermal infiltrate consisting mainly of lymphocytes and histiocytes. *A*, 48-hr reaction, Group III, control mouse. *B*, 48-hr reaction, Group I experimental mouse.

TABLE I. *Photosensitization to TCSA requires UVA at challenge*

Group	Day −3	Day 0	Day 1	Day 7	Day 8	Day 9
(6) I	Cy	TCSA +	TCSA +	TCSA-UVA	15.9 ± 4.2	12.8 ± 6.8
(6) II	Cy	UVB-UVA	UVB-UVA	TCSA	2.5 ± 1.4	1.7 ± 0.8
(6) III	—	—	—	TCSA-UVA	3.2 ± 3.9	2.5 ± 3.2
(6) IV	—	—	—	TCSA	0.8 ± 0.8	0.2 ± 0.4

Two groups (I and II) of 6 ICR female mice received cyclophosphamide (150 mg/k IP) on Day −3 and were sensitized by the application of 0.02 ml of 1% TCSA, followed in ½ hr by UVB-UVA to the skin of the left flank on Day 0 and Day 1. These mice and 2 toxicity control groups (III and IV) were challenged on the left ear on Day 7 as follows: Groups II and IV with 1% TCSA alone; groups I and III with 1% TCSA followed in ½ hr by UVA. The increase in ear thickness in mm × 10^{-2} ± SD is shown for each group at 24 and 48 hr.

Group I > Group III, $p < .001$ (24 hr readings).

larger reactions in Group III as compared with Group IV toxicity controls may represent the phototoxicity of TCSA; however, the difference was not statistically significant.

The histology of the 48-hr reactions from a representative animal of Group I and of Group III are shown in the Figure. The histopathology is consistent with allergic contact dermatitis induced in mice with strong contact allergens such as DNFB (dinitrofluorobenzene) without ultraviolet light [18].

Photo-ACD to TCSA could also be induced in inbred mice, although the sensitivity tended to be somewhat less intense than that seen in the ICR albino mice. For instance, in one experiment, CBA/J male mice were treated with cyclophosphamide (150 mg/k IP) on Day −3 and photosensitized with TCSA on Day 0 and Day 1. These mice, and a group of toxicity control mice were challenged on Day 7 with TCSA followed by UVA. Readings were made at 24 hr and 48 hr, and the challenge sites excised in 2 representative animals from each group after the 48 hr reading. The results are shown in Table II. We had comparable findings with the induction of photo-ACD to TCSA in Balb/c, C57Bl/6, C57Bl/6 × CBA/J, and DBA/2 mice. The histology of the photo-ACD reactions was comparable to that found in the ICR photosensitized mice. Successful photosensitization also requires UVR at the time of sensitization. In a typical experiment, different groups of ICR albino mice were putatively photosensitized to TCSA with and without further UVR. Only those mice that received UVR after allergen gave significant reactions at photochallenge. The protocol and results of this experiment are set down in Table III.

Induction of Photo-ACD to 6-Methylcoumarin and to Chlorpromazine

A group of 7 female ICR mice were clipped on the right flank and 0.02 ml of 1% 6-methylcoumarin in absolute alcohol was applied. Thirty minutes later the sensitization site was exposed successively to UVB (0.1 joules/cm^2) and to UVA (5.0 joules/cm^2). Day 1 the sensitization site was again treated with 6-methylcoumarin followed by UVR and the mice given 30 μg *C. parvum* intradermally into the sensitization site. A toxicity control group was similarly injected with *C. parvum* but not given photoallergen or UVR. On Day 6 both groups were challenged on the left ear with 1% 6-methylcoumarin in alcohol-corn oil (4:1) and the challenge site irradiated ½ hour later with UVA (5.0 joules/cm^2) (Table IV). Mice with a prior sensitizing exposure to 6-methylcoumarin and UVR showed significant reactions at photochallenge whereas toxicity control mice did not.

In several separate experiments, we photosensitized mice to chlorpromazine. In one such experiment, C57Bl/6 × CBA/J mice were sensitized on Day 0 and Day 1 to TCSA or to chlorpromazine by the application of 0.02 ml of either 1% TCSA or 1% chlorpromazine to the left flank followed 30 min later by exposure to UVR as stated previously. Challenge was made on Day 6 with both compounds followed by exposure to 5.0 joules/cm^2 UVA, the left ear being tested with 1% TCSA and the right with 1% chlorpromazine. Readings were made in the usual way at 24 hr and 48 hr. The specificity of the photosensitization was demonstrated; animals photosensitized to TCSA and tested with both compounds reacted just to the TCSA phototesting; whereas, those mice putatively photosensitized to chlorpromazine reacted only to photochallenge with that compound (Table V). In other experiments (not shown) with ICR mice, a substantially more intense photoallergic contact sensitivity to 1% chlorpromazine was induced with the same protocol.

Passive Transfer of Photocontact Allergy

Photo-ACD to TCSA could be transferred with regional lymph node cells from sensitized mice to naive mice of the same strain. The results of a typical experiment utilizing Balb/c mice

TABLE II. *Photosensitization of CBA/J mice to TCSA*

Group	Day −3	Day 0	Day 1	Day 7	Day 8	Day 9
I	Cy 150 mg/k	TCSA + UVB-UVA	TCSA + UVB-UVA	TCSA + UVA	12.0 ± 2.3	5.0 ± 0.6
II	—	—	—		1.3 ± 0.8	0.3 ± 0.5

Group I, consisting of 6 CBA/J male mice were treated with cyclophosphamide 150 mg/k IP on Day −3 and sensitized by the application of 0.02 ml of 1% TCSA to a skin site on the left flank, followed in ½ hr by UVB then UVA, on Day 0 and Day 1. Challenge was made to these mice, as well as to 6 toxicity control mice (Group II), on the ear with 1% TCSA followed by UVA. The 24-hr and 48-hr readings representing increase in ear thickness over baseline (mm × 10^{-2}) ± SD in each group are shown.

TABLE III. *Photo-ACD to TCSA requires UVA at sensitization*

Group	Day −3	Day 0	Day 1	Day 7	Day 8	Day 9
I	Cy	TCSA	TCSA	TCSA-UVA	2.3 ± 1.0	2.0 ± 0.5
II	Cy	TCSA + UVB-UVA	TCSA + UVB-UVA	TCSA-UVA	9.8 ± 1.7	8.0 ± 1.3
III	Cy	—	—	TCSA-UVA	2.3 ± 0.7	1.5 ± 0.3

Three groups of 6 ICR female mice received cyclophosphamide (Cy, 150 mg/k IP) on Day −3. Groups I and II received epicutaneously 0.02 ml of 1% TCSA on the skin of the left flank on Day 0 and Day 1; Group II mice were given, in addition, UVB-UVA ½ hr after each application. All mice were challenged on Day 7 with 1% TCSA followed in ½ hr by UVA. The increase in ear thickness in mm × 10^{-2} ± SD is shown for each group at 24 hr and 48 hr.

Group II > Group I, $p < .001$ (24-hr readings).

TABLE IV. *Photosensitization to 6-methylcoumarin*

Group	Day 0	Day 1	Day 6	Day 7	Day 8
I	6-MC + UVB-UVA	6-MC + UVB-UVA	6-MC + UVA	4.4 ± 1.4	5.6 ± 1.6
II	—	—		0.7 ± 0.8	0.7 ± 0.8

Fourteen ICR mice were divided into 2 groups. Group I mice received 0.02 ml of 1% 6 methylcoumarin to an area on the flank, followed by UVB (0.1 J/cm^2) and UVA 5 J/cm^2) on 2 successive days. On Day 6, these mice, as well as untreated mice of Group III, were tested on the left ear with 6-methylcoumarin (0.01 ml, 1%) followed by UVA (5 J/cm^2). 6-Methylcoumarin (0.01, 1%) was applied to the right ear immediately after the UVA. The average increase in left ear thickness (mm × 10^{-2}) of each group ± the standard deviation is shown. The increases in thickness of the right ear for Group I at 24 hr and 48 hr were 0.4 ± 0.8 and 0.6 ± 0.5; the comparable readings in Group II were 0.3 ± 0.5 and 0.3 ± 0.5.

Group I > Group II, $p < .001$ (left ears), at 24 hr.

TABLE V. *Specificity of photosensitization to TCSA and to chlorpromazine*

Group	Day 0	Day 1	Day 6	Day 7 TCSA-UVA	CPZ-UVA
I	TCSA + UVR	TCSA + UVR	TCSA-UVA	8.0 ± 3.6	0.9 ± 1.6
			and	(9.4 ± 4.4)	(1.6 ± 1.9)
II	CPZ + UVR	CPZ + UVR	CPZ-UVA	0.7 ± 1.0	3.2 ± 1.3
				(0.3 ± 0.8)	(4.3 ± 1.4)

Thirteen CBA × C57Bl/6 mice were divided into 2 groups. Group I had applied 0.02 ml of 1% TCSA to the skin on the flank, followed in 30 min by UVB and UVA, on Days 0 and 1. Group II mice were treated in parallel with 1% chlorpromazine (CPZ) followed by UVB and UVA on Day 0 and Day 1. On Day 6 these mice were challenged with 0.02 ml of 1% TCSA on the left ear and 1% chlorpromazine on the right ear and ½ hour later, both ears were irradiated with UVA. The average increase in thickness of the left and right ears, ± the standard deviation, at 24 hr and 48 hr ( ) is shown.

For TCSA-UVA challenge, Group I > Group II, $p < .001$; for CPZ-UVA challenge, group II > Group I, $p < .017$ (24-hr readings).

TABLE VI. *Passive transfer of photo-ACD to TCSA in Balb/c mice*

Group	Day −0	Day 1	Day 4	Day 4	Day 5	Day 6
I	TCSA-UVR	TCSA-UVR, *C. parvum*	Cell donor	—	—	—
II	TCSA-UVR	TCSA-UVR, *C. parvum*	—	TCSA-UVA	15.3 ± 2.1	12.0 ± 1.4
III	—	—	Cells from I	TCSA-UVA	6.6 ± 2.7	3.6 ± 1.3
IV	—	—	—	TCSA-UVA	1.5 ± 0.8	1.2 ± 1.0

Thirteen Balb/c mice were given 0.01 ml of 1% TCSA to the skin of each dorsal quadrant, followed by UVB-UVA, on Day 0 and Day 1. In addition, the mice received 30 μg of *C. parvum* intradermally into one sensitization site on Day 1. Day 4, nine of the actively sensitized mice (Group I) were sacrificed and a pool made of their regional lymph node cells. Each of 5 naive recipient Balb/c (Group III) mice were injected with 10^8 cells intravenously and ear tested ½-hour later with 1% TCSA followed by UVA (5 J/cm^2). Four positive controls (Group II) and 6 negative controls (Group IV) were ear tested in parallel. The increase in ear thickness (mm × 10^{-2}) at 24 and 48 hr ± the standard deviation is shown.

Group II > Group III, $p < .008$; Group III > Group IV, $p < .009$ (24-hr readings).

TABLE VII. *Photosensitization to TCSA of B cell deficient mice*

Group	Day 0	Day 1	Day 6	Day 7 TCSA-UVA (L)	UVA-TCSA (R)
I	TCSA-UVR	TCSA-UVR *C. parvum*	TCSA	11.5 ± 2.3	2.5 ± 1.4
			+		
II	—	*C. parvum*	UVA	2.5 ± 0.5	1.7 ± 1.2

Six C57Bl/6 × CBA/J mice that had been rendered B cell deficient (see Materials and Methods) received on the skin of the left flank 0.02 ml of 1% TCSA followed ½ hr later by UVB and UVA on Day 0 and Day 1. In addition, these mice as well as a further group of 6 B-cell deficient control mice of the same strain, were given 30 μg of *C. parvum* intradermally into the skin of the left flank. Day 6, both groups were challenged simultaneously on each ear with 1% TCSA, followed by (left ear) or preceded by (right ear) UVA (5 J/cm^2). The increase in ear thickness (mm × 10^{-2}) at 24 hr ± the standard deviation is shown.

Left ear: Group I > Group II, $p < .001$.

are shown in Table VI. Balb/c mice were photosensitized on Day 0 to TCSA by the application of 1% TCSA to each of four dorsal clipped quadrants followed by UVR (UVB + UVA). This routine was repeated on Day 1 and at that time 30 μg of *C. parvum* injected intradermally into one of the sensitization sites in each mouse after the UVR exposure. On Day 4, nine of the actively sensitized mice were sacrificed, the draining lymph nodes of the 4 quadrants excised and made into a cell suspension. 10^8 nucleated, trypan blue excluding, cells contained in a volume of 0.5 ml was injected intravenously into each of five recipients. One-half hr later these recipients as well as groups of toxicity control and actively sensitized mice were tested for photosensitivity to TCSA on the left ear. The results are shown in Table V. The donor animals were well-sensitized (Group II). Substantial sensitivity was also evident in the mice recipients of lymph node cells from the actively sensitized mice (Group III). Challenge sites were taken at 48 hr from representative mice in each of the 3 groups. The histological findings were consistent with those described for the passive transfer of allergic contact dermatitis in mice and in other animals. A single attempt to transfer with viable lymph node cells, photo-ACD to TCSA from Balb/c mice (H-2^D) to CBA/J mice (H-2^K) failed completely.

Induction of Photocontact Hypersensitivity in B-Cell Deficient Mice

A group of B-cell deficient mice were photosensitized to TCSA. These mice as well as a further group of six naive B-cell deficient mice were challenged on the ear with 1% TCSA followed by UVA. The results of this experiment are outlined in Table VII. B-cell deficient mice could be readily photosensitized to TCSA.

DISCUSSION

In experiments described herein we have demonstrated that mice can be photosensitized to TCSA, chlorpromazine, 6-methylcoumarin and, in preliminary findings, to sodium omadine. Clinically, all these chemicals are regarded as photocontact allergens. TCSA, which was once incorporated in soap as a germicide, was responsible for a remarkable epidemic of photo-ACD involving thousands of individuals in England twenty years ago. Because of its photosensitizing properties, its use was abandoned [19–21]. More recently, 6-methylcoumarin, which was widely used as a fragrance in a variety of topical preparations, was banned by the FDA from suntan preparations because it caused photo-ACD in a significant number of people [22,23]. The photosensitizing potential of TCSA, chlorpromazine and 6-methylcoumarin has been clearly demonstrated in humans by the "photomaximization" test [24]. Using this same experimental procedure, sodium omadine produced photocontact sensitivity in 6 of 25 tested volunteers (unpublished observations).

Prospective tests to identify photoallergic contact allergens have been proposed in humans and in the guinea pig [24–26]. The photomaximization test in humans appears to give results concordant with clinical experience. However, clearly it would be advantageous to do at least a preliminary screening of compounds for photoallergenicity in experimental animals. In addition, for a systematic study of the immunology of photo-ACD a genetically defined animal model is preferred; maneu-

vers, such as adoptive transfer, are more or less precluded in humans. Prospective testing of photoallergens has been done by several groups in the guinea pig. In a large-scale effort dealing with the phototoxicology and photoallergenicity of test compounds in the guinea pig, Maurer, Weirich, and Hess utilized complete Freund's adjuvant and multiple photosensitizing exposures of the chemical to induce sensitivity. In general, these investigators succeeded in identifying photocontact allergens of humans in the guinea pig. However, a curious lacuna was 6-methylcoumarin; in their test system, it was negative [27]. In the human photocontact allergy assay system, 6-methylcoumarin was a strong photocontact allergen, indeed, stronger than TCSA [24]. Recently, Ichikawa, Armstrong, and Harber reported that they had succeeded in sensitizing guinea pigs to 6-methylcoumarin by the addition of complete Freund's adjuvant to their sensitizing protocol [28]. Thus, it would appear from these preliminary studies that the mouse model may be at least an equally sensitive system for identifying potential photocontact allergens of humans.

What mechanisms are involved in photoallergy? Clearly the specificity of the reaction is changed by UVR. That the UVR must be given after, and not before allergen (e.g., Table VII), implies that its effect is that of photoactivation of the allergen and not merely that of a nonspecific irritant leading (say) to increased skin penetration of the allergen. Currently, one of our most important toxicity controls involves a double ear challenge in each test animal viz. allergen then UVA for one ear and UVA then allergen for the other. Very occasionally a weak sensitizer, such as TCSA, will give a single positive reaction in a group of test mice, as in the experiment outlined in Table I. Strong classical contact allergens such as DNFB or oxazolone sensitize essentially all mice in this system; however, in our experience (unpublished) relatively weak allergens such as formalin, picric acid or TCSA are generally non-sensitizing. Our experiments do not indicate *how* UVR renders the photoallergen allergenic, only that it does. In particular, on the basis of our present work we cannot decide whether UVR changes the photoallergen to a classical allergen, whether it facilitates the binding of photoallergen to carrier protein or whether one or more other mechanism is involved [1,29]. Solution of this problem is for the future.

Stephan Epstein was the first to suspect that photosensitivity could be immunologically mediated [30]. He made this observation following a photosensitization experiment in which he induced photoreactivity in human volunteers by the intradermal injection of sulfanilamide followed by UVR. It is interesting that in the human photo-maximization test, which employs exclusively the topical administration of presumptive allergen, sulfanilamide was scored as a nonphotosensitizer [24]. The photosensitizing potential of some systemically administered drugs may therefore not be detected with epicutaneous testing.

The experiments of Sams (*vide infra*) appear to show an *in vitro* correlate of *in vivo* photo-ACD [3]. The passive transfer of TCSA photo-ACD in guinea pigs and the demonstration of an *in vitro* response of peritoneal exudate cells from TCSA-photosensitized guinea pigs to a TCSA-albumin-UVR-reacted complex provides evidence that photo-ACD is a delayed-type hypersensitivity analogous to conventional ACD [3,6]. However, it is curious that albumin was a successful carrier protein for the photoallergen TCSA in the *in vitro* test, since with classical contact allergens, there is strong evidence that the sensitizer conjugates with proteins on the surface of Langerhans cells and that these cells function as antigen-presenting cells [31]. Guinea pigs contact sensitized to DNCB do not react to DNP-albumin [32]. Rats and mice have been used for the study of phototoxicity; up to now, investigations dealing with photo-ACD in these species have been lacking [26].

Ultraviolet light has been shown to be uniquely toxic to Langerhans cells and to other antigen presenting cells [33,34]. Yet photoallergic reactions occur. In one study, there was a delay of at least 12 hr between UVB exposure and the loss of the surface markers of Langerhans cells [35]. This interval may be sufficient for the antigen presentation of photoallergens, or, perhaps, other mechanisms may explain the failure of ultraviolet irradiation to prevent the induction of photoallergy. Experiments such as pre-irradiation of the sensitization site, should help to clarify this issue.

During the past 12 yr studies of classical contact allergens in the mouse have provided us with an enormous amount of fundamental information concerning the immunoregulation of cell-mediated hypersensitivity. The model of photo-ACD in mice that we propose is a variant of that which is standard for the study of ACD in mice to classical contact sensitizers such as DNFB and oxazolone. Operationally, the additional steps required for the induction and challenge are the irradiation of the sensitization site with UVR and of the challenge site with UVA. Although in the experiments reported herein we have utilized UVB during induction, preliminary experiments suggest that irradiation with UVA alone is sufficient. This would agree with the findings of Horio that photo-ACD can readily be induced in guinea pigs using UVA without UVB if the sensitization site is pretreated with an irritant such as sodium lauryl sulfate [36].

We were able to photosensitize inbred as well as randomly bred mice. This allows for investigations into regulatory mechanisms of photo-ACD that are modeled after the now classical studies that utilize immunogenetic manipulations of conventional ACD to strong allergens in mice.

The clinical course of photo-ACD in the mouse is that of a delayed-type hypersensitivity reaction viz. the challenge reaction is negligible at 4 hrs. (data not shown) and significant at 24 and 48 hr. The histology of the challenge reactions are consistent with those of allergic contact dermatitis to conventional contact allergens in the mouse. To examine the role of B cells and B cell products in the reaction of photo-ACD, we used mice that had been rendered B cell deficient by the chronic administration from birth of a goat anti-sera directed against mouse IgM. This model of the B-cell deficient mouse has been used in our own and in other laboratories in different contexts [13–16]. We found that B-cell deficient mice can be readily photocontact sensitized to TCSA. This implies that the specificity for the photocontact allergy is given by a population of T cells. Supporting this notion, we found that adoptive transfer of photo-ACD was readily accomplished from actively sensitized Balb/c mice to naive Balb/c mice utilizing regional lymph node cells. These results parallel the passive transfer of conventional ACD in mice and guinea pigs [37,38]. The subpopulation of lymphoid cells responsible for the transfer of photo-ACD remains to be characterized. In a single attempt, we failed to transfer photo-ACD from Balb/c ($H\text{-}2^D$) to CBA/J ($H\text{-}2^K$) mice. Transfer of standard ACD in mice requires H-2 compatibility [39].

Killed *C. parvum* injected into the sensitization site heightens the acquisition of photo-ACD in mice (data not shown). We have had a similar experience with the immunopotentiation of conventional ACD by *C. parvum* in mice, rats, hamsters and guinea pigs [40–42]. Cyclophosphamide pretreatment is widely used for the immunopotentiation of conventional ACD in mice and other species [43,44]. We routinely used cyclophosphamide as an immuno-adjuvant in our early experiments with photoallergens in mice. However, it must be emphasized that substantial photoallergy in mice can be induced *without* the use of immunopotentiators (see Tables IV and V). In some cases, where more strongly sensitized animals are desired, as in the case of donors for cellular transfer experiments (Table VI), an immunological adjuvant is a convenience. Further, it may be that immunopotentiators are required for the induction of photo sensitization with relatively weak photoallergens, just as they are required in many bioassays for the identification of weak classical contact sensitizers in the guinea pig [45].

In summary, we have established a mouse model for photo-ACD. This model should be useful for determining the mech-

anisms that regulate photo-ACD. In addition, the mouse may prove to be a proper test animal for the bioassay of putative photocontact allergens of humans.

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